fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF to generate rat neural crest stem cells.

REMARKS

Claims 1, 9-13 and 15-18 are pending in the instant application. Claims 1, 9-13 and 15-18 have been rejected. Claims 9-13 and 16 have been canceled, without prejudice. Claims 1 and 15 have been amended. No new matter has been added by these amendments. Reconsideration is respectfully requested in light of the following amendments and remarks.

I. Rejection of Claims 1, 9-13 and 15-18 under 35 U.S.C. § 112, first paragraph

Claims 1, 9-13 and 15-18 have been rejected under 35 U.S.C. § 112, first paragraph. The Examiner has acknowledged the specification to be enabling for the method of plating dissociated cells on a fibronectin substrate and a purification step of obtaining "pure, homogeneous populations of neuroepithelial cells, etc." However, the Examiner suggests that the specification does not reasonably provide enablement for a method requiring "isolating a pure, homogeneous population of mammalian neural stem crest cells" by replating neuroepithelial

cells onto laminin-coated or undefined substrates while removing FGF and/or chick embryo extract.

Applicants respectfully traverse this rejection.

In accordance with MPEP § 2164.08, all questions of enablement must be evaluated against the claimed subject matter. Claim 1 of the instant application, as well as claim 15, are drawn to methods for generating mammalian neural crest stem cells. These methods comprise obtaining mammalian neuroepithelial stem cells derived from the neural tube from a mammalian embryo at a stage of embryonic development after closure of the neural tube and inducing the neuroepithelial stem cells to differentiate *in vitro* by replating the neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF. There are no steps in claims 1, 9-13 or 15 and 16 involving "isolating a pure, homogeneous population of mammalian neural stem crest cells". Nor are there steps for "replating neuroepithelial cells onto laminin-coated or undefined substrates while removing FGF and/or chick embryo extract". Accordingly, the subject matter which the Examiner has identified as lacking enablement is not relevant to the instant claims as this subject matter is not set forth in the

pending claims.

Further, while new claims 17-18 are drawn methods for isolating mammalian neural crest stem cells, the steps comprise generating neural crest stem cells in accordance with the method of claim 1 and isolating the neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75. Accordingly, the Examiner's suggestion of subject matter not enabled by the instant specification is also irrelevant to these claims.

Teachings of the specification which are relevant and enabling for the claimed subject matter can be found at 19-21 and pages 50-63 of the instant specification.

Specifically with respect to claims 1 and 15, beginning at page 19, detailed methods are set forth for obtaining mammalian NEP cells as claimed in step (a) of claim 1. At page 19, lines 6-11, it is taught that a sample of neural tube is removed after closure of the neural tube (claim 1, step (a), part (i)). At page 19, lines 22-29, a method for dissociating cells as set forth in claim 1, step (a), part (ii), is taught. Further, plating of the dissociated cells on a substratum in a media comprising FGF and CEE as claimed in claim 1, step (a) part (iii) is taught in the specification at pages 20-21, Examples 2 and 3.

Harvesting of the NEP cells by trypsinization prior to differentiation as set forth in step (b) of the claim 1 and 15 is taught at page 51, lines 15-17 of the specification. A method for inducing the NEP cells to differentiate *in vitro* by replating the neuroepithelial stem cells onto a fibronectin substrate in neural crest media in accordance with step (c) of claim 1 is described beginning at page 51, line 14. The components of neural crest media as defined in step (c) of claim 1 are taught in the specification at page 50, lines 18-21.

For claims 17-18, step (b) of isolating the neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75 is described in detail in Example 19 beginning at page 52 of the application.

Thus, the specification clearly enables one of skill to make and use the invention as set forth in independent claims 1, 15 and 17-18 and therefore meets the requirements of 35 U.S.C. § 112, first paragraph.

With respect to claims 9-13 and 16, it is respectfully pointed out that these claims have been canceled, without prejudice, thus mooting this rejection as it pertains to these claims.

Withdrawal of this rejection under 35 U.S.C. § 112, first

paragraph, is therefore respectfully requested.

II. Rejection of Claims 9 and 16 under 35 U.S.C. § 112, second paragraph

Claims 9 and 16 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner suggests that the metes and bounds of the recitation "dorsalizing agent" remains ambiguous.

It is respectfully pointed out that claims 9 and 16 have been canceled, thus mooting this rejection.

Withdrawal of this rejection under 35 U.S.C. § 112, second paragraph, is therefore respectfully requested.

III. Rejection of Claims 1, 9, 15 and 16-18 under 35 U.S.C. § 102(e)

Claims 1, 9, 15 and 16-18 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Anderson et al. (U.S. Patent 5,589,376) and by Anderson et al. (U.S. Patent 5,824,489). The Examiner has re-instated this rejection because of the amendment of the claims to remove the recitation of "isolating a pure, homogenous population . . ." Further, the Examiner suggests

that, absent evidence to the contrary, the inclusion of EGF, bFGF and NGF (e.g. col. 12 of '376; col. 156 of '489) or the addition of forskolin (col. 16 of '376; col. 18 of '489) in the culture medium of the Anderson patents meets the limitations of "adding a dorsalizing agent".

Applicants respectfully traverse this rejection.

At the outset, it is respectfully pointed out that claims drawn to adding a dorsalizing agent have been canceled. Accordingly, the Examiner's suggestion that the inclusion of EGF, bFGF and NGF or addition of forskolin as taught by Anderson et al. meets the limitations of "adding a dorsalizing agent" is irrelevant to the pending claims.

Further, the pending claims have been amended to clarify that the neuroepithelial stem cells are harvested by trypsinization prior to replating the neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF to generate neural crest stem cells. Support for the amendment can be found in the specification at page 51, lines 15-17.

The Andersen patents do not teach a method with these steps. Instead, the Andersen patents teach:

(1) plating of the neural tube onto fibronectin plates;

(2) culturing the plated neural tubes for 24 hours during which time neural crest cells migrate away from the neural tube onto the substrate;

(3) scraping the neural tube away from the neural crest cells using a Tungsten needle; and

(4) harvesting the crest cells from the plate via trypsinization. See specifically column 13, line 59, through page 14, line 31 of U.S. Patent 5,824,489 and column 8, line 29-50 of U.S. Patent 5,589,376. Since the Andersen patent do not teach the steps for obtaining and harvesting neuroepithelial stem cells prior to inducing the cells to generate neural crest stem cells, these patents cannot anticipate the invention as now claimed.

Withdrawal of this rejection under 35 U.S.C. § 102(e) is therefore respectfully requested.

IV. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made

to the specification and claims by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,

/Kathleen A. Tyrrell/

Kathleen A. Tyrrell
Registration No. 38,350

Date: **October 30, 2002**

LICATA & TYRRELL P.C.
66 E. Main Street
Marlton, New Jersey 08053

(856) 810-1515

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel claims 9-13 and 16, without prejudice.

Please amend claims 1 and 15 as follows:

1. (amended) A method for generating mammalian neural crest stem cells comprising:

(a) obtaining mammalian neuroepithelial stem cells derived from the neural tube from a mammalian embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a mammal at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the mammal; and

(iii) plating the dissociated cells in feeder-cell-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract so that mammalian neuroepithelial stem cells are obtained; ~~and~~

(b) harvesting the mammalian neuroepithelial stem cells by trypsinization; and ~~inducing the neuroepithelial stem cells to differentiate in vitro by~~

(c) replating the neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF~~, thereby generating said~~ to generate neural crest stem cells.

15. (amended) A method for generating rat neural crest stem cells comprising:

(a) obtaining rat neuroepithelial stem cells derived from the neural tube from a rat embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a rat at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the rat; and

(iii) plating the dissociated cells in feeder-cell-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract so that rat neuroepithelial stem cells are obtained; ~~and~~

(b) harvesting the rat neuroepithelial stem cells by trypsinization; and ~~inducing the neuroepithelial stem cells to differentiate in vitro by~~

(c) replating the rat neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF~~, thereby generating said~~ to generate rat neural crest stem cells.